Glucocorticoid programming of intrauterine development

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ABSTRACT

Glucocorticoids are important environmental and maturational signals during intrauterine development. Towards term, the maturational rise in fetal glucocorticoid concentrations decreases fetal growth and induces differentiation of key tissues essential for neonatal survival. When cortisol levels rise earlier in gestation as a result of suboptimal conditions for fetal growth, the switch from tissue accretion to differentiation is initiated prematurely, which alters the phenotype that develops from the genotype inherited at conception. While this improves the chances of survival should delivery occur, it also has functional consequences for the offspring long after birth. Glucocorticoids are, therefore, also programming signals that permanently alter tissue structure and function during intrauterine development to optimise offspring fitness. However, if the postnatal environmental conditions differ from those signalled *in utero*, the phenotypical outcome of early life glucocorticoid overexposure may become maladaptive and lead to physiological dysfunction in the adult. This review focusses on the role of glucocorticoids in developmental programming, primarily in farm species. It examines the factors influencing glucocorticoid bioavailability *in utero* and the effects that glucocorticoids have on the development of fetal tissues and organ systems, both at term and earlier in gestation. It also discusses the windows of susceptibility to glucocorticoid overexposure in early life together with the molecular mechanisms and long term consequences of glucocorticoid programming with particular emphasis on the cardiovascular, metabolic and endocrine phenotype of the offspring.

1. Introduction

Glucocorticoids are important stress hormones in adult animals but have a wider range of functions in the fetus. In late gestation, they act as maturational signals that ensure the fetus is mature enough to survive the transition to extra-uterine life at delivery [1]. Earlier in gestation, glucocorticoids can act as signals of suboptimal environmental conditions and modify fetal development in relation to the available resource for intrauterine growth. While improving the likelihood of survival both *in utero* and at birth, this early overexposure to glucocorticoids adapts the phenotype that develops from the genotype inherited at conception with life-long functional consequences [2-11]. Glucocorticoids are, therefore, also programming signals that permanently alter tissue structure and function during intrauterine development to optimise offspring fitness [12, 13]. Previous reviews of glucocorticoid programming have tended to concentrate on the human implications and/or the experimental studies of short lived, laboratory species like mice, rats and

guinea pigs [2-9]. In contrast, this review examines the role of glucocorticoids in developmental programming with particular emphasis on the longer-lived farm species like sheep, pigs, cattle and horses.

2. Fetal glucocorticoid exposure

There are a number of different mechanisms by which glucocorticoid concentrations can rise in the fetal circulation (Figure 1). For most of gestation, the primary source of cortisol in fetal ovine plasma is the mother [19]. Glucocorticoids cross the placenta readily by diffusion down a materno-fetal concentration gradient which exists in normal conditions in all species studied to date including pigs, cattle, sheep, pigs and horses [1,3]. Consequently, increases in maternal glucocorticoid concentrations induced by stressful conditions, such as isolation, transport, undernutrition and housing conditions, can lead to raised concentrations in the fetus [20]. However, the degree of fetal overexposure to the higher maternal glucocorticoid concentrations is minimised by the presence in the placenta of the enzyme, 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2, Figure 1). This isoform of the enzyme converts active glucocorticoids into their inactive keto-metabolites and, hence, acts as a barrier to transplacental glucocorticoid transfer [14]. Amongst species, placental 11βHSD2 activity appears to be positively related to the magnitude of the materno-fetal glucocorticoid gradient and is influenced by gestational age and a range of environmental factors including glucocorticoid concentrations on both sides of the placenta (Figure 1). In addition, in the hemochorial type of placenta, there are multidrug resistance transporters, which transfer xenobiotics like synthetic glucocorticoids from the trophoblast cells back into the maternal circulation (Figure 1). Abundance of these transporters are also regulated developmentally and by glucocorticoids but whether they are present in the epitheliochorial placenta of ruminants, pigs and horses remains unknown [9]. Fetal glucocorticoid concentrations can, therefore, be altered independently of maternal levels by manipulating the effectiveness of the placental barrier to materno-fetal glucocorticoid transfer. Glucocorticoid programming in early to mid-gestation, therefore, depends on the level of stress experienced by the mother during pregnancy, her HPA responses and ensuing cortisol concentration, and on the glucocorticoid permeability of the placenta.

Later in gestation when the fetal hypothalamic-pituitary-adrenal (HPA) axis has developed functionally, fetal glucocorticoid concentrations can also rise independently of maternal levels by

direct cortisol secretion from the fetal adrenal glands (Figure 1). This occurs via activation of the HPA axis in response to adverse intrauterine conditions like hypoxia and hypoglycaemia caused, for example, by cord occlusion, placental insufficiency, poor uterine perfusion or maternal alterations in dietary composition or calorie intake [20]. Development of fetal HPA responsiveness to adverse stimuli varies in timing between species and with both early glucocorticoid overexposure and repeated insults during late gestation [13,21]. Closer still to term, fetal cortisol concentrations rise naturally in the absence of adverse stimuli as part of the normal sequence of prepartum maturational events that ensure viability at birth [1; Figure 2]. The magnitude and timing of this normal prepartum cortisol surge also varies widely between species (Figure 2) and can be activated earlier than normal by poor nutritional conditions either around the time of conception or during late gestation [1,18,22,24]. Its timing is also influenced by the number of fetuses in sheep [22]. In some species like the horse, the main perinatal rise in cortisol concentrations occurs immediately after not before birth [21, 25]. The window of susceptibility to glucocorticoid programming in late gestation will, therefore, vary with species in relation to environmental conditions *in utero* and the development and responsiveness of the fetal HPA axis.

Fetal glucocorticoid overexposure can also occur as a result of clinical use of synthetic glucocorticoids like dexamethasone and betamethasone during pregnancy. These drugs are 20 times more potent than the natural hormones and are cleared more slowly from the circulation [26]. They are often used to treat conditions with an inflammatory component such as joint and respiratory problems, allergic reactions and endotoxic shock in several species [26-28]. They are also given routinely to healthy pregnant women threatened with pre-term delivery to improve neonatal viability of their infants [26]. Since the onset of labour is co-ordinated with maturation through the prepartum cortisol rise in many ruminants, synthetic glucocorticoids are also used to induce delivery of viable offspring at or near term in cattle and sheep [29-31]. Experimentally, these drugs have been used extensively in the longer-lived farm species to investigate the likely long-term physiological consequences for the human infant of antenatal glucocorticoid treatment [10,11].

The developmental effects of the glucocorticoids are determined ultimately by their bioavailability within the tissues. In turn, this depends on expression of the glucocorticoid (GR) and mineralocorticoid receptors (MR) to which the glucocorticoids bind [14]. These receptors vary in abundance between fetal tissues and with gestational age [32-34]. Their expression can also be influenced by glucocorticoid concentrations *per se* [35]. In addition, fetal tissues express 11βHSD, both the type 2 isoform found in the placenta and the type 1 isoform which reactivates the

biologically inactive metabolites [14]. Activity of the two isoforms varies between tissues and both isoforms are regulated developmentally and by fetal oxygen, nutrient and glucocorticoid availability in a tissue specific manner [32-35]. Consequently, overexposure to glucocorticoids is determined not only systemically by the circulating concentrations but also locally within the tissues themselves. Since synthetic glucocorticoids are poorly inactivated by 11βHSD2 and bind only to the GR [14], their bioavailability and actions can differ from those of the natural glucocorticoids.

3. Glucocorticoid programming

3.1 *Glucocorticoids and fetal development*

Maturationally, glucocorticoids affect development of a wide range of fetal tissues, particularly those essential for immediate neonatal survival like the lungs, liver, gut and kidneys [1,13]. During late gestation, experimental manipulation of fetal cortisol concentration in fetal sheep by fetal adrenalectomy and exogenous cortisol infusion have shown that cortisol induces changes in tissue expression of cytostructural proteins, receptors, enzymes, ion channels and growth factors [1,3]. These changes lead to alterations in tissue morphology, biochemical composition, metabolism and hormone sensitivity with functional consequences for multiple organ systems in the fetus. For example, in addition to their well established role in pulmonary maturation [1,26], glucocorticoids increase fetal blood pressure during late gestation via effects on the fetal heart and blood vessels [36]. Similarly, the fetal liver develops the capacity of glucogenesis close to term as a result of cortisol-induced increases in glycogen deposition and gluconeogenic enzyme activities [37]. Glucocorticoids, therefore, activate many of the physiological processes that have little or no function *in utero* but which are vital at birth like pulmonary gas exchange, hepatic gluconeogenesis and thermogenesis [1]. They also affect development of many other tissues like the brain and skeletal muscle which are important for offspring viability and fitness in the longer term [38, 39]. Consequently, delivery before adequate intrauterine exposure to rising cortisol concentrations leads to functional immaturity at birth, poor neonatal viability and/or a failure to thrive postnatally [40]. This scenario is likely to be particularly important in twin-bearing and polytocous species like sheep and pigs in which the timing of fetal HPA activation can differ between littermates.

The maturational effects of the glucocorticoids are mediated, in part, by changes in the circulating concentrations and tissue bioavailability of a range of other hormones [41]. In fetal sheep, the functioning of several endocrine systems including the HPA axis itself is affected by the prepartum

cortisol surge via changes in endocrine cell populations, enzyme activities and hormone receptor abundance (Figure 3). This leads to prepartum increases in fetal plasma concentrations of several hormones in addition to cortisol, including tri-iodothyronine (T_3), leptin and adrenaline [20]. In turn, these hormones have independent effects on development of a range of fetal tissues [41]. For instance, terminal differentiation of mononucleated cardiac myocytes to their binucleated form is initiated by the prepartum cortisol surge but depends on activation of specific tissue deiodinases and the concomitant increase in fetal T_3 bioavailability [42,45]. The changes in the set point and sensitivity of the endocrine axes induced by the prepartum cortisol surge also prepare the fetus for the new homeostatic challenges of extrauterine life. For example, the cortisol induced increases in the adrenal activity of phenylethanolamin-N-methyl transferase (PNMT) and the hepatic abundance of β-adrenoreceptors mean that neonates can secret adrenaline in response to stressful conditions like hypoglycaemia and respond to the circulating adrenaline and produce glucose endogenously [37,46,47].

Early increases in the fetal glucocorticoid concentration also trigger tissue differentiation in the fetus [1,13]. However, in sheep, the effects of preterm cortisol administration do not entirely recapitulate the maturational changes in tissue differentiation induced by the increase in cortisol concentrations towards term. For example, adrenal PNMT abundance is increased by the prepartum cortisol surge but is decreased by cortisol administration at 100 days of gestation [46,48]. Similarly, cortisol depresses hepatic IGF-II expression at term but not earlier in gestation [49]. The transcriptome observed in the fetal lung and heart after early cortisol infusion also differs from that seen at term [50,51]. This probably reflects, in part, the ontogenic changes in tissue abundance of GR and/or other hormone receptors.

By simulating tissue differentiation, cortisol reduces tissue accretion *in utero* [1,13]. As a result, the overall rate of fetal growth declines as cortisol concentrations rise in fetal sheep towards term and in response to adverse intrauterine conditions [13]. The prepartum decline in growth rate can be prevented by fetal adrenalectomy and can to be stimulated prematurely by infusing cortisol into either the fetus or mother earlier in gestation [13,52]. In several species including laboratory and farm animals, maternal administration of synthetic glucocorticoids in late gestation has also been shown to reduce offspring size, both shortly after administration and at delivery longer after the period of treatment [26,53,54; Table 1]. Similar reductions in fetal growth have been seen with administration of synthetic glucocorticoids directly to fetal sheep although the effects appear to be less pronounced than with maternal administration [53]. This suggests that the growth inhibitory

effects of synthetic glucocorticoids may be mediated, in part, by maternal metabolic changes or actions on the placenta [10, 103].

3.2 Glucocorticoids and placental development

Reductions in placental weight are seen in response to administration of both natural and synthetic glucocorticoids during mid to late gestation in sheep and other species [104]. These changes are associated with reduced expression of anti-apoptotic markers and increased expression of pro-apoptotic factors in the ovine placenta [105]. The growth inhibitory effects are more pronounced with maternal than fetal administration and tend to persist after cessation of treatment [105]. They may also be sex-linked [10]. In sheep, both maternal and fetal cortisol administration alter the gross placental morphology with proportionately fewer of the more everted placentomes [106,107]. Although the functional significance of this shift in placentome distribution remains unclear [106,108,109] placentas with fewer everted C and D type placentomes transport more glucose on a weight specific basis when fetal cortisol concentrations are high [106]. In general, glucocorticoids reduce placental glucose transport via effects on the transplacental glucose concentration gradient, placental glucose consumption and/or placental expression of the glucose transporters, dependent on the species [104]. They also reduce the active transport of amino acids across the placenta and alter placental amino acid metabolism in some species [104]. Glucocorticoid-induced changes in placental transport phenotype also vary with time both during the period of treatment and after it has ended [104]. In addition, there are alterations in the endocrine function of the placenta in response to raising glucocorticoid concentrations, which involve a wide range of hormones and changes in both their synthesis and metabolism (Figure 2). Again, these effects can be sex-linked and are often dependent on gestational age at the time of glucocorticoid exposure. For example, placental 11βHSD2 gene expression is increased by dexamethasone administration to ewes at 30% of gestation in males alone but decreased by treatment later in pregnancy in both sexes [10,105,109].

The changes in placental endocrine and transport function induced by the prepartum cortisol surge are part of the normal sequence of events leading to labour and delivery of viable neonates [1,40]. However, their induction by glucocorticoid overexposure earlier in gestation may alter fetal growth and development independently of any direct effects of the glucocorticoids on the fetal tissues *per se* [1,3,13]. For example, the reduction in placental lactogen production and its maternal concentration in response to early glucocorticoid overexposure may alter maternal metabolism and, hence, nutrient allocation to the gravid uterus [109]. Similarly, glucocorticoid induced changes in

the production of progesterone and other progestagens may influence maternal insulin resistance and appetite with indirect consequences for intrauterine growth [110]. In addition, changes in placental phenotype induced by early glucocorticoids overexposure may persist or appear only after restoration of normal concentrations to affect fetal development long after original insult [104].

3.3 Postnatal outcomes of early overexposure to glucocorticoids

Glucocorticoid exposure *in utero* has been shown to affect organ growth and the functioning of physiological systems in the offspring after birth in sheep, pigs and cattle (Table 1). In particular, there are abnormalities in postnatal cardiovascular and metabolic function that are associated with overt hypertension and glucose intolerance by adulthood. These changes involve a wide range of tissues including the brain, blood vessels, kidneys, heart and skeletal muscle as well as several endocrine systems (Table 1). Similar findings have been made in adult rodents and humans overexposed to glucocorticoids prenatally [2-9]. Because of the central role of glucocorticoids in regulating adult cardiovascular and metabolic function, many of these studies have concentrated on programming of the HPA axis *per se* (Table 1). In sheep, pigs and cattle, early prenatal overexposure to either natural or synthetic glucocorticoids can alter both basal and stimulated cortisol concentrations postnatally and, hence, the physiological responses to homeostatic challenges (Table 1). Indeed, amongst species, maternal glucocorticoid administration during late pregnancy has been shown to programme postnatal HPA function at every level of the axis from the hippocampus to glucocorticoid bioavailability in the peripheral tissues [11]. Taken together, these studies have shown that glucocorticoids overexposure *in utero* affects the same range of tissues and cellular processes in the adult as seen in the fetus [1,3,13]. However, the specific postnatal outcomes of intrauterine glucocorticoid overexposure depend on gestational age at its onset, its severity and duration and on whether exposure was to natural or synthetic glucocorticoids (Table 1).

Natural and synthetic glucocorticoids have different programming effects. Maternal administration of cortisol but not dexamethasone at 20% of ovine pregnancy causes fasting hyperglycaemia while dexamethasone but not cortisol increases their initial insulin response to glucose administration in the adult male offspring [53]. In contrast, both cortisol and dexamethasone administration at this stage of pregnancy give rise to hypertension in the adult offspring [61]. However the mechanism by which hypertension is induced differs with cortisol increasing peripheral resistance but not cardiac output while dexamethasone enhances cardiac output but not peripheral resistance in the adult

sheep [4]. Different synthetic glucocorticoids also appear to have different programming effects (Table 1), although few, if any, studies have specifically compared the postnatal consequences of dexamethasone and betamethasone treatment *in utero*.

In sheep, glucocorticoids have been shown to have programming effects on postnatal phenotype with administration from as early as 27 days of pregnancy right up until term (Table 1). However, the specific outcomes depend on gestational age at onset of treatment (Table 1). For instance, maternal administration of a single course of dexamethasone leads to hypertension in the adult offspring when given at 27 and 80 days but not at 64 days of pregnancy [63,64]. In contrast, dexamethasone has little effect on glucose tolerance or insulin sensitivity of adult female offspring with administration at either 27 days or 64 days of gestation [64]. Similarly, maternal dexamethasone treatment early in pregnancy appears to have little effect on HPA function but, later in gestation, it decreases HPA responsiveness of the adult offspring [69,111]. Furthermore, multiple doses of betamethasone over a 14-d period in late pregnancy have subtly different effects on HPA function and glucose-insulin dynamics of the adult offspring than single doses given at the same gestational age as that at the start of the more prolonged treatment [86,87]. Longer periods of dexamethasone treatment at lower doses also have little effect relative to a single treatment at the higher, clinically relevant doses during the same period of gestation [4,112]. Consequently, the dose of synthetic glucocorticoid administered as well as its duration and timing in pregnancy is important in determining the phenotypical outcome.

Another factor influencing the apparent extent of programming is the postnatal age at which the outcomes are assessed (Table 1). Some glucocorticoid-programmed changes in postnatal growth and physiological function are apparent immediately after birth while others only become evident later in life as the animal ages, reaches key life course events like weaning, puberty or pregnancy or experiences adverse conditions after birth [113; Table 1]. For example, hypertension is not seen in the neonatal lamb after intrauterine dexamethasone overexposure, although there are changes in the baroflexes indicative of resetting of the neural mechanisms of blood pressure control even at this early stage of postnatal life [56]. Hypertension is evident at 4 months of age at about the time weaning is complete and becomes progressively more pronounced with increasing age thereafter [57]. Overall, the experimental studies suggest that glucocorticoid-programmed metabolic dysfunction appears later in ovine life than the cardiovascular abnormalities and is often not detected until adulthood (Table 1). Metabolic changes may also only be detected in one sex (Table

1). For instance, altered glucose-insulin dynamics are seen in 4-5 year-old male but not female offspring overexposed to dexamethasone at 27 days of gestation [55,64]. In addition, there is emerging evidence in rodents and other species that maternal diet and pre-existing conditions such as intrauterine growth restriction can influence the feto-placental responses to glucocorticoid administration, which, in turn, are likely to affect programming of postnatal phenotype [114,115].

3.4 Developmental windows of glucocorticoid programming

Collectively, the experimental studies summarised in Table 1 suggest that there are specific stages in development when glucocorticoid overexposure is most likely to result in an altered postnatal phenotype. The first window of susceptibility is probably during pre-implantation development when lineage specification occurs and cells are segregated into trophectoderm and inner cell mass. Certainly, undernutrition during this period of pregnancy has effects on development of the fetal HPA axis and other organ systems much later in gestation [18,24]. The second vulnerable period for glucocorticoid programming is during organogenesis which occurs between days 7 and 30 of gestation in sheep embryos. This also covers the period of implantation and formation of the ovine placenta [10]. Indeed, compromised development of the metanephric kidney is likely to be a significant contributory factor in the hypertension seen in adult offspring of ewes treated with dexamethasone at 27 days of gestation [4]. After completing organogenesis, there is a relatively long period of gestation when the fetus is gaining mass and developing the neural and endocrine mechanisms regulating homeostasis. During this period, excess glucocorticoids appear to act by changing the kinetics of the cell cycle to slow growth and set the responsiveness of the regulatory mechanisms. When tissues have developed sufficient GR or at critical concentrations or duration of exposure, glucocorticoids can switch the cell cycle from proliferation to differentiation prematurely, with permanent effects on total cell number and/or the balance of different cell types within an organ [13]. For example, cortisol induced differentiation of cardiomyocytes from the mononucleated form, which can divide, to the binucleated type, which cannot, means that cell number is fixed and that cardiac growth depends primarily on cell hypertrophy rather than hyperplasia thereafter [42]. Finally, in some species, there appears to be a window of susceptibility to glucocorticoid programming in the period immediately after birth, which may be particularly important in species like the horse in which terminal differentiation of tissues is not complete at birth [21]. Neonatally, cortisol overexposure may occur either directly due to sickness or maladaptation *ex utero* or indirectly via changes in milk composition and its glucocorticoid content as a result of maternal stress or abnormal mammary development [116,117]. Certainly,

experimental overexposure of healthy newborn foals to cortisol for 5 days after birth alters both HPA and pancreatic β cell function later in life (Table 1).

3.5 Molecular mechanisms of glucocorticoid programming

At the molecular level, there appears to be two broad mechanisms by which glucocorticoids act to programme development. First, bound to their receptors, they may act as enhancer binding proteins that activate or repress expression of genes via interaction with glucocorticoid response elements in the promotor or other regulatory regions of the genome [118]. With genes that trigger key developmental stages, their altered expression at inappropriate times in the normal sequence of events may have permanent effects on the subsequent pattern of development. This type of glucocorticoid-induced change in expression of specific genes may occur either early in development, for example during cell lineage specification and mitochondrial biogenesis, or later in gestation during differentiation of sub-populations of cells within tissues such as the liver or endocrine pancreas. The outcomes of these discrete gene expression events may, therefore, be global in terms of cell metabolism and oxidative stress or specific to certain tissues or cell types. In rodents, increased oxidative stress is a common feature of glucocorticoid programming along with changes to the relative numbers of the different endocrine cell types within the Islets of Langerhans [2-9].

Secondly, glucocorticoids may alter the epigenome with more long term consequences for gene expression throughout life [119]. Changes in DNA methylation and histone modifications have been observed both globally and in specific tissues in postnatal offspring glucocorticoid overexposed *in utero* [35,119]. In particular, there are tissue specific changes in the methylation status of the regulatory regions of the GR gene which influence expression of these receptors and, hence, postnatal glucocorticoid responsiveness [33, 118]. Maternal dexamethasone administration during pregnancy has been shown to reduce placental transport of folate required for one carbon metabolism and DNA methylation while, conversely, dietary supplementation with folate ameliorates, in part, the feto-placental growth restriction induced by this treatment [115,121]. In addition, there is emerging evidence for postnatal changes in expression of various non-coding and microRNAs after early life glucocorticoid overexposure [39]. Glucocorticoids, therefore, affects the developing epigenome through a number of different routes with dynamic consequences for epigenetic marks throughout the lifespan of the animal. However, to date, most of the information

about the molecular mechanisms of glucocorticoid programming has been derived from studies in rodents and guinea pigs so the extent to which they apply to farm species remains unclear.

4. Conclusions

Glucocorticoids have a number of roles during intrauterine and early neonatal development. Not only are they essential for normal maturation close to term, they also act as important signals of environmental compromise earlier in gestation. The glucocorticoid triggered switch from tissue accretion to differentiation improves offspring fitness by maximising the chances of the fetus surviving into adulthood. Prenatally, early activation of this switch ensures that fetal growth is commensurate with the nutrient supply *in utero* and that fetal tissues are sufficiently mature to function *ex utero* should delivery occur. Postnatally, the glucocorticoid-induced adaptations in phenotype and, particularly the resetting of the homeostatic control mechanisms, will help the offspring to thrive in a postnatal environment matching that signalled to it *in utero*. However, when pre- and post-natal environments are mismatched in laboratory species, the glucocorticoid-induced changes in offspring phenotype can become maladaptive and lead to early onset of cardiometabolic dysfunction characteristic of old age [2-9]. Recent findings have also shown that the effects of early life glucocorticoid overexposure can persist inter-generationally with changes in F2 placental phenotype and HPA function after dexamethasone treatment of their pregnant grandmothers [122-125]. This raises the possibility that glucocorticoids may also have an important evolutionary role in the transgenerational inheritance of phenotypical traits. However, the extent to glucocorticoid overexposure during early development influences lifespan and transgenerational inheritance in longer lived farm species remains largely unknown.

5. References

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Figure legends

Figure 1: Schematic diagram showing the sources of cortisol in the fetal circulation and the role of 11 beta-hydroxysteroid dehydrogenase as a placental barrier to materno-fetal cortisol transfer in sheep. Open arrows = major cortisol movements. Dashed arrow = minor cortisol movement. → Stimulatory effect. ⊣ Inhibitory effect.
Data from references 14-18.

Figure 2: Fetal cortisol concentrations with respect to proximity to delivery in different species. Length of pregnancy: Pig 115 days (filled circles), Sheep 145 days (open circles), Human 280 days (filled triangle), Cow 280 days (filled squares), Horses (Pony) 335 days (open triangles). Data from references 1,22,23.

Figure 3: The endocrine systems affected by natural and synthetic glucocorticoids in fetal sheep together with the cellular and molecular processes within these endocrine systems influenced by prenatal glucocorticoid exposure. Data from references 1-11,42-44.

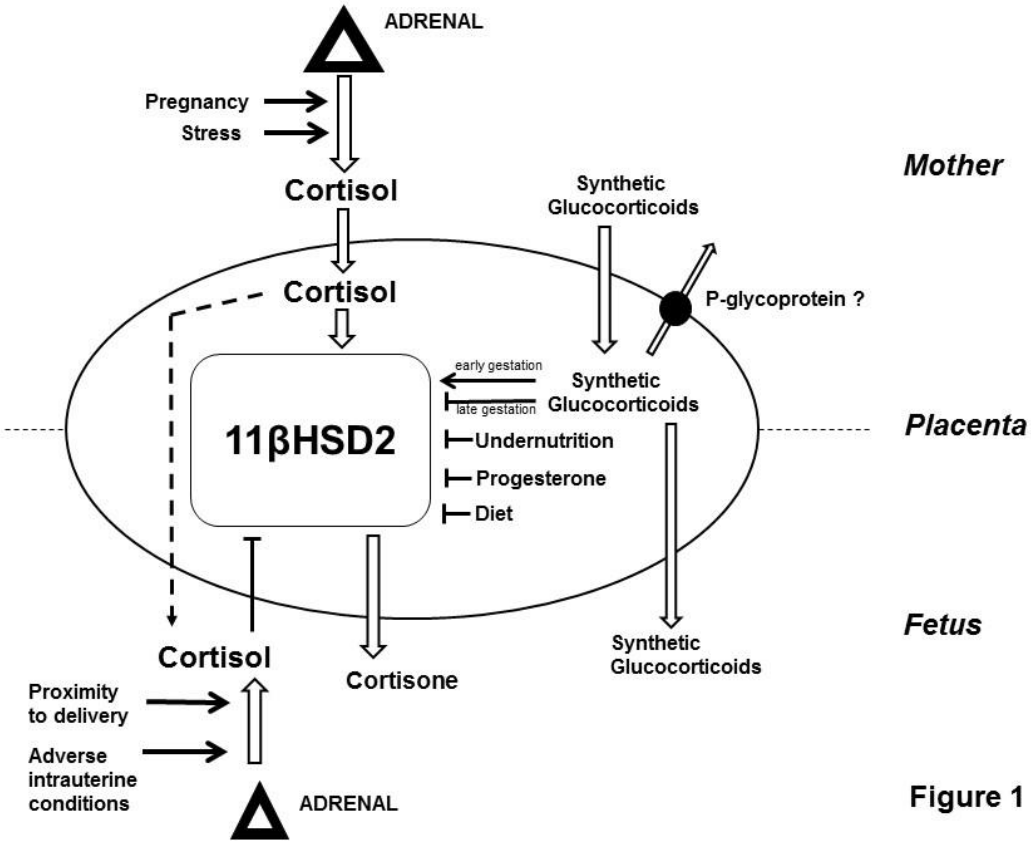
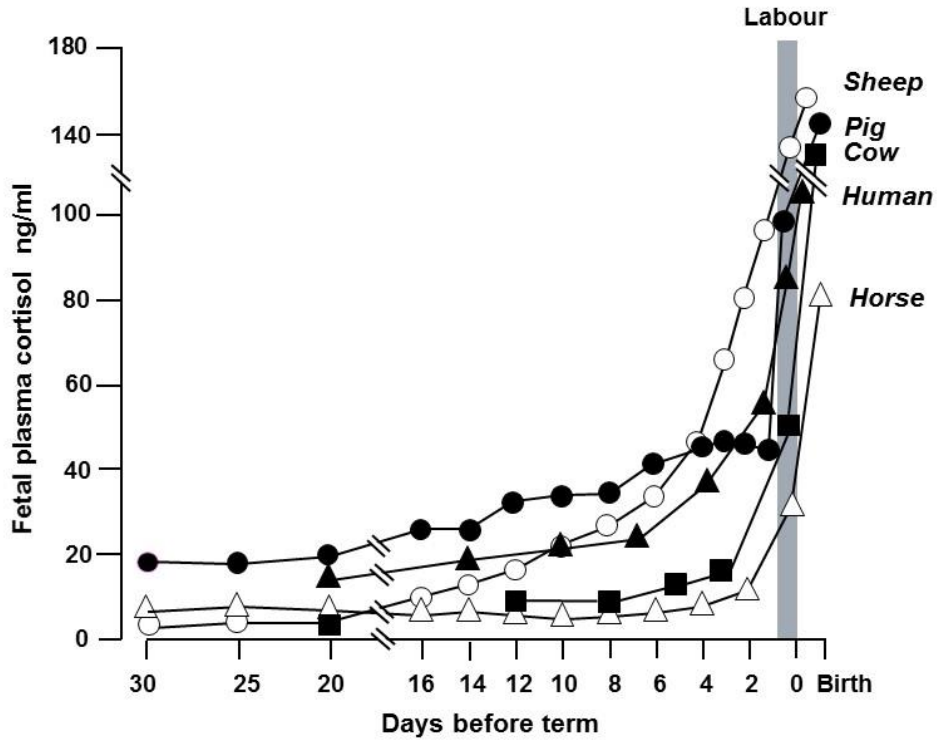
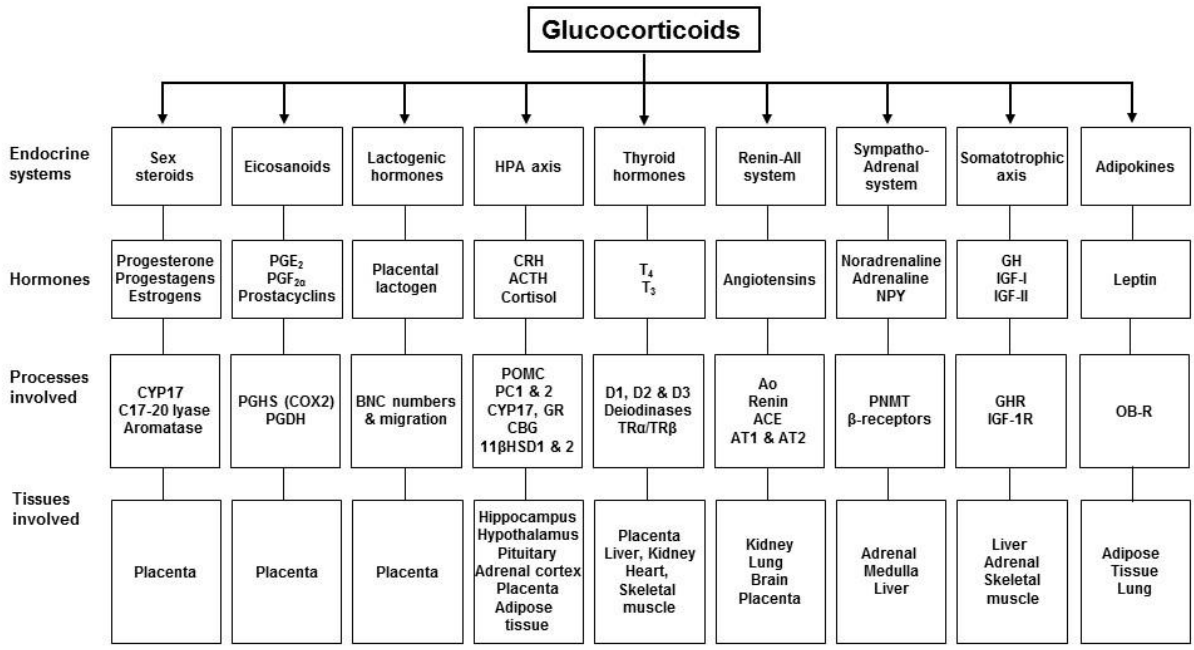

ADRENAL
Pregnancy
Stress
Cortisol
Mother
Synthetic
Glucocorticoids
Cortisol
P-glycoprotein ?
early gestation
late gestation
Synthetic
Glucocorticoids
11βHSD2
Undernutrition
Progesterone
Diet
Placenta
Fetus
Cortisol
Cortisone
Synthetic
Glucocorticoids
Proximity
to delivery
Adverse
intrauterine
conditions
ADRENAL

Figure 1

Figure 2


Labour
Sheep
Pig
Cow
Human
Horse
Fetal plasma cortisol ng/ml
180
140
100
80
60
40
20
0
30
25
20
16
14
12
10
8
6
4
2
0
Birth
Days before term


Glucocorticoids
Endocrine systems
Hormones
Processes involved
Tissues involved
Sex steroids
Eicosanoids
Lactogenic hormones
HPA axis
Thyroid hormones
Renin-AII system
Sympatho-Adrenal system
Somatotrophic axis
Adipokines
Progesterone Progestagens Estrogens
PGE2 PGF2α Prostacyclins
Placental lactogen
CRH ACTH Cortisol
T4 T3
Angiotensins
Noradrenaline Adrenaline NPY
GH IGF-I IGF-II
Leptin
CYP17 C17-20 lyase Aromatase
PGHS (COX2) PGDH
BNC numbers & migration
POMC PC1 & 2 CYP17, GR CBG 11βHSD1 & 2
D1, D2 & D3 Deiodinases TRα/TRβ
Ao Renin ACE AT1 & AT2
PNMT β-receptors
GHR IGF-1R
OB-R
Placenta
Placenta
Placenta
Hippocampus Hypothalamus Pituitary Adrenal cortex Placenta Adipose tissue
Placenta Liver, Kidney Heart, Skeletal muscle
Kidney Lung Brain Placenta
Adrenal Medulla Liver
Liver Adrenal Skeletal muscle
Adipose Tissue Lung

827

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829

Table 1: The postnatal outcome of early life overexposure to glucocorticoids in farm animals.							
	Species	Agent	Stage of pregnancy at overexposure	Postnatal outcomes	Age at outcome N = Neonate J = Juvenile A = Adult	Sex of Offspring M = Male F = Female	Reference
Maternal Synthetic Glucocorticoid Overexposure	Sheep	Dex	20%	↓Birth weight and abdominal circumference	N	M	55
				Altered baroreflexes, ↑sympathetic activity	N	M & F	56
				Altered vasodilatory responses	N	M & F	56
				Hypertension	J (4 mo) A (1.5-2 yr) A (5-6 yr) A (7 yr)	M & F M & F F M & F	57 58 59 60
				Left ventricular hypertrophy ↑Cardiac output	A (7 yr) A (7 yr)	F F	58 58
				Altered endothelial superoxide production	J (4 mo)	M & F	61
				Altered cardiac mitochondrial function	J (6 mo)	M	62
				Altered brain RAS function ↑brainstem AT1 receptor abundance	A (4-5 yr) A (7 yr)	M F	63 60
				↓Nephron number ↑Mean glomerular volume ↑single nephron glomerular filtration rate	A (5-6 yr) A (7 yr) A (7 yr) A (5-6 yr)	F M & F M & F F	59 58 60 59
				↑Glucose stimulated insulin secretion ↑Glucose tolerance	A (4 yr) A (4 yr)	M M	54 54
				↑sensitivity to inhibition of lipolysis by insulin	A (5 yr)	F	64
			30%	↑pituitary-adrenal responsive to stress ↓liver, adrenal, pituitary & kidney weight ↓pituitary-adrenal responsiveness ↑Hippocampal GR mRNA ↑Hypothalamic AVP & GR mRNA ↓Pituitary POMC mRNA	N (30 d) J (7 mo) J (7 mo) J (7 mo) J (7 mo) J (7 mo)	F not M F F not M M not F M not F F not M	65 66 67 67 67 67

				↑Adrenal ACTH receptor, StAR and 3βHSD mRNA	J (7 mo)	M not F	67
			70%	↓body weight and CRL	N	F	68
				↓basal and stimulated HPA function	A (2.5-3.5 yr)	F	69
				↓Glucose tolerance	A (2.5-3.5 yr)	F	69
				↓Insulin secretion	A (2.5-3.5 yr)	F	69
			70-82%	↓ brain weight and size	N	M & F	54
			98%	↑UCP1 content in brown adipose tissue	N	M & F	70
				↑Prolactin receptor abundance	N	M & F	70
				↑Relative fat mass	A (16 mo)	F not M	71
		Beta	55%	↑sympathetic and HPA responses	J (40 d)	F	72
				↑basal and ACTH stimulated cortisol secretion	A (1.5 yr)	F not M	73
				Hypertension	J (6 mo)	M & F	74
					A (1-2yr)	M	75
				Altered systemic and renal RAS function	J (6 mo)	M	76
				↓plasma renin and AII concentrations	J (6 mo)	M & F	77
				↑AII stimulated ROS production	J (6 mo)	M & F	76
				Altered renal AII responsiveness	J (6 mo)	M & F	78
					A (1-1.5 yr)	M & F	79
				↓nephron number	J (6 mo)	M & F	80
				↓glomerular filtration rate	J (6 mo)	M	80
				Altered cerebral vascular tome and reactivity	A (1.5 yr)	F	81
				↑plasma leptin	A (1.5 yr)	M & F	73
				↑ leptin inhibition of adrenal function	A (1.5 yr)	M not F	73
			72-84%	↓Brain weight	J (6 wk)	M & F	66
					A (3.5 yr)	M & F	82
				↓Lung weight	J (12 wk)	M & F	66
				↓testicular development	J (6 & 12 wk)	M	83
				↓Body weight	J (12 wk)	M & F	84
				Hypotension	J (12 wk)	M & F	82
				↓plasma T_3 levels	J (6 & 12 wk)	M & F	66
				↓plasma IGF-I & IGFBP levels	J (12 wk)	M & F	85

				↓hypothalamic AVP & CRH mRNA	J (6 & 12 wk)	M & F	65
				↓pituitary POMC, PC1 & PC2 mRNA	J (6 & 12 wk)	M & F	65
				↓ pituitary CRH/AVP responsiveness	J (7 mo)	F not M	67
				↓Pituitary GR	J (7 mo)	M & F	67
				↑basal and ACTH stimulated cortisol levels	A (1 yr)	M & F	86
				↑pituitary CRH/AVP responsiveness	A (2 yr)	M & F	86
				↓adreno-cortical ACTH responsiveness	A (3 yr)	M & F	87
				↓basal ACTH and cortisol levels	A (3 yr)	M & F	87
				↓plasma glucose levels	J (12 wk)	M & F	65
				Insulin resistance	J (6 mo)	M & F	84
				Glucose intolerant	A (1.5 yr)	M & F	84
				↑Glucose stimulated insulin secretion	A (1.5 yr)	M & F	84
				↑Fasting insulin:glucose ratio	A (2 & 3 yr)	M & F	88
				↑Hepatic glucose-6-phosphatase activity	A (3.5 yr)	M & F	88
	Horse	Dex	95%	↓Body weight	N	M & F	89
				↓ CRL and adreno-cortical ACTH responsiveness	N	M & F	90
Maternal Cortisol Overexposure	Sheep	Cortisol	20%	↑renal Na^+-K^+ ATPase α-subunit	J (2 mo)	M & F	59
				↓glomerular number	A (4-5 yr)	F	59
				↑single nephron GFR	A (4-5 yr)	F	59
				Hypertension	A (1.5 yr)	M & F	63
					A (4-5 yr)	F	59
				Fasting hyperinsulinaemia	A (4 yr)	M	54
				↑Glucose stimulated insulin secretion	A (4 yr)	M	54
			50-72%	↓Body weight	N	M & F	90
		Periodic isolation	72%-term	↑Birth weight	N	M & F	92
				↑Basal Cortisol concentration	N	M & F	92
	Pig	Cortisol	33-50%	↑body weight	J (5 mo)	M	93
		ACTH	40%	↑basal LH	N	F	94
			40-65%	↑plasma CBG & ↑adrenomedullary cells	N	M & F	95
			40-73%	↑adrenal cortex:medulla ratio	N & J (60 d)	M & F	96
				↑Hypothalamic CRH and adrenal ACTH receptor	N	M & F	96
				↓Hypothalamic endorphin	J (30 d)	M & F	96

				↑Pituitary POMC mRNA	J (60 d)	M & F	96
				↑HPA stress responsiveness	J (11 wk)	F	96
			75-93%	↓Body weight	N & J	M & F	95
				↑plasma CBG & 5HT	N & J	M & F	95
				↓Relative adrenal weight ↑adrenal cortex area	J (4 wk)	M & F	95
				Altered brain neurotransmitter system	J (4 wk)	M & F	95
		Social mixing	35-56%	↑hypothalamic CRH expression to social stress	J (9 wk)	F	97
				↑Cortisol response to social stress	J (9 wk)	F	97
			67-97%	↑Cortisol response to social stress	J (9 wk)	F	97
	Cow	ACTH	20-50%	↑Body weight	N (at birth)	M & F	98
				↑Cortisol secretion to restraint	J (5 mo)	M & F	98
		Transport	20-50%	↑Cortisol secretion to restraint	J (5 mo)	M & F	98
				↓Cortisol clearance	J (5 mo)	M & F	98
				↑Heart rate increment to restraint	J (5 mo)	M & F	98
Fetal Synthetic Glucocorticoid Overexposure	Sheep	Beta	72-84%	↑Glucose-stimulated insulin secretion	J (6 mo)	M & F	83
				Glucose intolerant	A (1 yr)	M & F	83
				↓Basal insulin concentration	A (2 yr)	M & F	88
				↑Basal insulin concentration	A (3 yr)	M & F	88
				↑Hepatic glucose-6-phosphatase activity	A (3.5 yr)	M & F	88
				↓ Pituitary CRH/AVP responsiveness	A (1 yr)	M & F	86
				↑ Adreno-cortical ACTH responsiveness	A (1 yr)	M & F	86
				↓Basal and stimulated ACTH concentration	A (2 yr)	M & F	87
				↑ Adreno-cortical ACTH responsiveness	A (2 yr)	M & F	87
				↓Brain weight	A (3.5 yr)	M & F	82
Neonatal Glucocorticoid Overexposure	Sheep	Dex	3-4 days	Altered NMDA receptor kinetics	5 d	M & F	99
	Horse	ACTH	1-5 days	↓Glucose stimulated insulin secretion	J (2 & 12 wk)	M & F	100
				↑Basal cortisol concentrations	J (12 wk)	M & F	101
				Altered pituitary sensitivity to hypoglycaemia	A (1 & 2 yr)	M & F	102

Dex=dexamethasone, Beta=betamethasone, ACTH=Adrenocorticotrophic hormone, AII=Angiotensin II, AT1=Angiotensin receptor type 1, AVP=Arginine vasopressin, CBG=Corticosteroid binding globulin, CRH=Corticotropin releasing hormone, CRL=Crown rump length, GR= Glucocorticoid receptor, GRF=glomerular filtration rate, HPA=hypothalamic-pituitary-adrenal, 3βHSD=hydroxysteroid dehydrogenase, 5HT=5-hydroxytryptamine, IGF-I=Insulin-like growth factor I, IGFBP=Insulin-like growth factor binding protein, LH=Luteinising hormone, NMDA=N-methyl-D-aspartate, POMC=Pro-opiomelanocortin, RAS=Renin-angiotensin system, ROS=Reactive oxygen species, StAR=Steroidogenic acute regulatory protein, T_3=Tri-iodothyronine, UCP1=Uncoupling protein 1.

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